BULL. BOT. SURV. INDIA
Vol. 27, Nos. 1-4 : pp. 142-144, 1985

INFLUENCE OF INORGANIC NITROGEN-NUTRIENTS ON COPPER TOXICITY IN *ANACYSTIS NIDULANS* AND CYANOPHAGE AS-1 RESISTANT MUTANT

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ABSTRACT

The effect of different nitrogen sources on growth and copper toxicity was studied in the unicellular cyanobacterium *Anacystis nidulans* and Cyanophage AS-1 resistant mutant (An/AS-1). The mutant An/AS-1 was characterised by slow growth rate but was more senstitive to copper than its parent. In both the strains, the presence of high nitrate concentrations, which supported better growth of algae, reduced the algicidal effect of copper. However, other inorganic nitrogen sources such as nitrite and ammonium caused more inhibition and reduced the specific growth rate.

INTRODUCTION

An important environmental problem which is beginning to assume major proportions is that of the toxic essential and non-essential metals. Among essential trace elements copper salts are widely used as agricultural algicides and its requirements in trace amounts for many physiological processes of cyanobacteria have been emphasized (Sorentino, 1979). Many laboratory studies (Fitzgerald, 1964, Hutchinson and Stokes, 1975) have suggested that the factors affecting copper toxicity may be classified as : those which affect the concentration of toxic ionic form of copper and those which affect the physiology of algae. Its effects on various physiological processes have been studied in detail (Kashyap and Gupta, 1982 a, b, Gupta, 1983, 1986). It has also been observed that unicellular cyanobacteria, in general, were relatively more sensitive than the filamentous forms (Gupta *et al.*, 1985). Here the toxic effect of copper on *Anacystis nidulans* and cyanophage AS-1 resistant mutant (An/AS-1) under the influence of different nitrogen sources is discussed.

MATERIAL AND METHOD

Axenic culture of *Anacystis nidulans* (ATCC 27144) and its cyanophage AS-1 resistant mutant (An/AS-1) was maintained in Allen's medium (Allen, 1968) with A-5 trace elements (devoid of copper). Culture conditions and frequency of isolation of An/AS-1 were described elsewhere in detail (Gupta, 1983).

Exponentially grown cultures (8 days) were washed by repeated centrifugation (4,000 × g, 10 min) and resuspended in the medium. Graded concentration of copper was added as $CuSO_4 \cdot 5H_2O$ (BDH, India). Cultures without copper served as control. Growth was monitored colorimetrically at 650 nm. The specific growth rate (k) was calculated using the equation given by Myres and Kratz (1955).

RESULTS AND DISCUSSION

Specific growth rate (k) in absence or presence of graded concentrations of copper (Table 1) revealed that growth was inhibited in all the concentrations of copper employed but the general pattern of growth remain unchanged. The mutant An/AS-1 was found to be more sensitive (lethality at 4 μM) than its parent (lethality at 5 μ M). It

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Date of receipt : 9.7.86. Date of acceptance : 11.12.86

Table 1 : Effect of different nitrogen sources on copper toxicity (expressed in terms of *k*)

Strains	Cu^{++}conc. (μM)	NO_3(mM)				NH_4(mM)		NO_2(mM)	
		5	10	15	20	1	5	1	5
A. nidulans	Control	0.32	0.40	0.46	0.42	0.42	0.40	0.19	0.25
	1.0	0.21	0.35	0.45	0.42	0.38	0.35	0.15	0.20
	2.0	0.09	0.28	0.38	0.40	0.28	0.22	0.12	0.13
	5.0	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00
An/AS-1	Control	0.30	0.38	0.41	0.40	0.40	0.38	0.18	0.20
	1.0	0.19	0.32	0.40	0.40	0.36	0.30	0.13	0.15
	2.0	0.09	0.27	0.30	0.38	0.25	0.17	0.08	0.10
	5.0	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00

might be due to higher rate of copper uptake by the mutant than its parent (Kashyap and Gupta, 1982 b, Gupta, 1986). Analogous to the present observation it has been reported earlier that other algae are also susceptible to copper within the range of 4-8 μM (Fitzgerald, 1964, Gupta and Arora, 1978, Kashyap and Gupta, 1980). The finding that at a concentration of 4 μM the lag phase in *A. nidulans* was approximately two times longer than the control (graph not shown) confirms the earlier finding of Laube *et al.* (1980) for *Anabaena*. A long lag in presence of copper may be interpreted as a net result of adaptation and death of cells.

In both the strains 1-2 μM of copper served as an algistatic in the presence of high nitrate concentrations (15-20 μM) but becomes algicidal in low nitrate value (<15 μM) in the aquatic environment. It revealed that the presence of high nitrate concentrations which supported better growth of algae, reduced the algicidal effect of copper. The presence of other inorganic nitrogen nutrients may affect the growth response of cells in the presence of copper. Low *k* values were observed in both the strains when $NaNO_3$ was replaced either by $NaNO_2$ or NH_4Cl in the medium. However, the *k* value increased as the nitrite concentration increased from 1-5 mM. The response of both the strains to different levels of NH_4Cl remained insensitive, however, the *k* value were significantly lower than the nitrate or nitrite supplemented medium. The results clearly indicated that irrespective of the strains used in the present investigation, other inorganic nitrogen sources caused more inhibition than nitrate. The site(s) at which such inhibition occurs remain unknown.

ACKNOWLEDGEMENTS

I thank Dr. R. S. Safferman, Univ. of Cincinnati, Ohio, USA, for samples of *Anacystis nidulans* and Cyanophage AS-1.

Thanks are also due to the Director, Botanical Survey of India for encouragements and to Mr. A. R. K. Sastry for going through the manuscript.

REFERENCES

ALLEN, M. M. Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.* 4 : 1-4. 1968.

FITZGERALD, G. P. Factors in the testing and application of algicides. *Appl. Microbiol.* 12 : 247-253. 1964.

GUPTA, A. B. AND A. ARORA. Morphology and physiology of *Lyngbya nigra* with reference to copper toxicity. *Physiol. Plantarum* 44 : 215-220. 1978.

GUPTA, S. L. Acid and alkaline phosphatase activity in the cyanobacterium *Anacystis nidulans* under copper stress. *Folia Microbiologica.* 28 : 458-462. 1983.

GUPTA, S. L. Copper uptake and inhibition of growth, photosynthetic pigments and macromolecular synthesis in *Anacystis nidulans*. *Photosynthetica*. 20 : 75-80. 1986.

—— A. K. KASHYAP AND A. P. SINGH. Responses of blue-green algae to copper : A comparative study' *Indian J. Environ. Hlth.* 27(3) : 224-229. 1985.

HUTCHINSON, T. C. AND P. STOCKS. Heavy metal toxicity and algal bioassays. In : Water Quality Parameters. American Society for Testing Materials, STP No. 573, Philadelphia, pp. 320-343. 1975.

KASHYAP, A. K. AND S. L. GUPTA. Toxic effect of copper sulphate on two blue-green algae. *J. Sci. Res.* 30 : 97-104. 1980.

—— AND —— Pleiotropic behaviour of a cyanophage AS-1 resistant mutant of *Anacystis nidulans*. *Mol. Gen. Genet.* 185 : 365-366. 1982a.

—— AND —— Effect of lethal copper concentrations on nitrate uptake, reduction and nitrite release in *Anacystis nidulans*. *Z. Pflanzenphysiol.* 107 : 289-294. 1982b.

Laube, V. M., C. N. MCKENZIE AND D. J. KUSHNER. Strategies of response of copper, cadmium and lead by a blue-green and a green alga. *Can. J. Microbiol.* 26 : 1300-1311. 1980.

MYRES, J. AND W. A. KRATZ. Relations between pigment content and photosynthetic characteristics in a blue-green algae. *J. Gen. Physiol.* 39 : 11-92. 1955.

SORENTINO, C. The effects of heavy metals on phytoplankton—a review. *Phycos.* 18 : 149. 1979.